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Forum Review

Impact of Mitochondrial ROS Production in the Pathogenesis of Diabetes Mellitus and Its Complications

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ABSTRACT

In this review, the impacts of mitochondrial reactive oxygen species (ROS) on diabetes and its complications are described. In endothelial cells, high-glucose treatment increases mitochondrial ROS and normalization of the ROS production by inhibitors of mitochondrial metabolism, or by overexpression of UCP-1 or MnSOD, prevents glucose-induced activation of PKC, formation of AGE, and accumulation of sorbitol, all of which are believed to be the main molecular mechanisms of diabetic complications. Glomerular hyperfiltration, one of the characteristics of early diabetic nephropathy, may be caused by mitochondrial ROS through activation of COX-2 gene transcription, followed by PGE_2 overproduction. In pancreatic β cells, hyperglycemia also increases mitochondrial ROS, which suppresses the first phase of glucose-induced insulin secretion, at least in part, through the suppression of GAPDH activity. In liver cells, similar to that in hyperglycemia, TNF-α increases mitochondrial ROS, which in turn activates apoptosis signal-regulating kinase 1 (ASK1) and c-jun NH_2-terminal kinases (JNK), increases serine phosphorylation of IRS-1, and decreases insulin-stimulated tyrosine phosphorylation of IRS-1, leading to insulin resistance. These results suggest the importance of mitochondrial ROS in the pathogenesis of diabetes mellitus and its complications through modification of various cellular events in many tissues, including vessels, kidney, pancreatic β cells, and liver. *Antioxid. Redox Signal. 9*, 343–353.

INTRODUCTION

THE MITOCHONDRION is believed to be an organelle derived from a genetic component(s) of microorganisms, and thus its replication, transcription, and translation system has been developed on its own basis, although several nuclear genome–encoded proteins are also essential for these systems.

Because mitochondria are the primary source of ATP production, disruption of mitochondrial respiratory function is regarded as one of the key factors in the development of several diseases, including Friedreich ataxia, Parkinson disease, Huntington disease, pathophysiology of aging, and diabetes and its complications. A mitochondrial mutation was found to be associated with maternally inherited diabetes mellitus (46). The mitochondrial dysfunction in islet β-cells was reported to impair insulin secretion in response to increased glucose concentration (30, 88, 95). Impaired mitochondrial oxidative phosphorylation in liver and muscle has been shown to be linked with insulin resistance or type 2 diabetes in humans (65, 66). Diabetes mellitus induced in rats by streptozotocin or alloxan treatment or in cats by pancreatectomy has been reported to impair mitochondrial respiration and disturb energy production in liver, skeletal muscle, heart, and diaphragm, and mitochondrial protein synthesis and function were restored to normal by administration of insulin (26, 27, 70, 87, 91). Thus, mitochondrial dysfunctions are deeply involved in the pathophysiology of diabetes.

Conversely, a number of *in vitro* and *in vivo* studies suggest that oxidative stress is increased in diabetic patients and animal models of diabetes (6, 20, 25, 32, 61, 74, 82, 94). Oxidative stress may be crucial for the pathogenesis of diabetic mellitus and its complications, and mitochondria have been reported to be the major source of reactive oxygen species (ROS).

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In this review, a possible involvement of ROS from mitochondrial electron transport chain in the development and progression of diabetic complications is demonstrated. In addition, our recent studies about the relations between mitochondrial ROS production and the pathogenesis of diabetes mellitus, including glucose toxicity in pancreatic β-cell and insulin resistance in insulin target organs is introduced.

ROLE OF MITOCHONDRIAL ELECTRON TRANSPORT CHAIN IN HYPERGLYCEMIA-INDUCED ROS PRODUCTION

The most universal and critical mitochondrial function is oxidative phosphorylation. The overall system of oxidative phosphorylation includes five large multienzyme complexes, designated complexes I, II, III, IV, and ATP synthase (Fig. 1). This mitochondrial respiratory system is thought to be the major source of ROS under normal physiologic conditions (8, 80). Two main sites of superoxide generation exist in this system: NADH dehydrogenase at complex I (89), and the interface between CoQ and complex III (12). The hypothesis that hyperglycemia could increase production of ROS from the mitochondrial electron transport chain has been studied.

To determine the involvement of the mitochondrial electron transport chain in hyperglycemia-induced ROS production, the effect of agents that alter mitochondrial metabolism on hyperglycemia-induced intracellular ROS formation was evaluated in bovine aortic endothelial cells. Using fluorescent probe DCF as a detector of ROS, increased ROS production compared with baseline conditions was confirmed in endothelial cells incubated with high (30 m*M*) glucose. Conversely, complex II inhibitor (thenoyltrifluoroacetone: TTFA) and an uncoupler (carbonyl cyanide m-chlorophenylhydrazone: CCCP), as well as overexpression of either uncoupling protein-1 (UCP1) or manganese superoxide dismutase (MnSOD), the mitochondrial form of antioxidant enzyme, prevented the effect of hyperglycemia (51, 59). Increased ROS production from mitochondria under a hyperglycemia condition was also detected by the fluorescence of the reduced form of MitoTracker Red, which specifically accumulates inside mitochondria, and that was prevented by overexpression of UCP1 and MnSOD (54). These results suggested that ROS produced from the mitochondrial electron transport chain was increased in a hyperglycemic condition *in vitro*.

Conversely, multiple pathways have been reported as the cause of oxidative stress in diabetes. These include enhanced auto-oxidation of glucose (41) and Amadori product (40); and activation of NADPH-dependent aldose reductase (polyol pathway), which diminishes the NADPH available for glutathione reductase, so the ratio of reduced to oxidized glutathione decreases (64, 93); stimulation of cellular ROS production by extracellular advanced glycation end products (AGEs) via their receptors (100); and protein kinase C (PKC)-dependent activation of NAD(P)H oxidase (44). Fur-

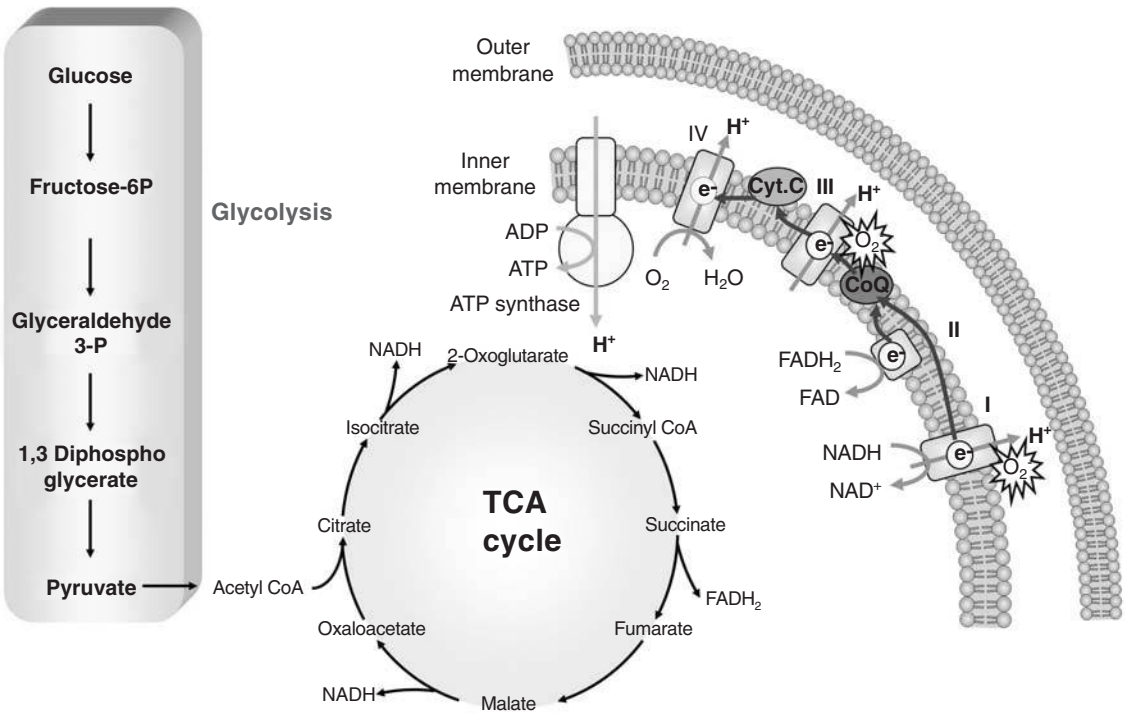

FIG. 1. Production of reactive oxygen species by the mitochondrial electron-transport chain. Oxidative phosphorylation includes five large multienzyme complexes, designated complexes I, II, III, IV, and ATP synthase. The mitochondrial respiratory system may be the major source of ROS under normal physiologic conditions.

ther study will be required to examine the relation between mitochondrial ROS production and the other mechanisms.

A SINGLE ELEMENT LINKING HYPERGLYCEMIA-INDUCED DAMAGE

Four main hypotheses about how hyperglycemia causes diabetic complications have generated a large amount of data, as well as several clinical trials based on specific inhibitors of these mechanisms. The four hypotheses are as follows: activation of the polyol pathway (55); increased AGEs formation (10); activation of PKC (44, 53); and activation of hexsosamine pathway (17) (Fig. 2). Until recently, no unifying hypothesis linked these four mechanisms. To examine whether mitochondrial ROS production could be one such unifying mechanism, the effects of inhibitors of mitochondrial ROS on these independent biochemical pathways were investigated. Similar to the previous reports, we confirmed that hyperglycemia activated PKC in membrane fraction, increased intracellular AGE formation, and increased sorbitol accumulation in bovine aortic endothelial cells. Conversely, mitochondrial ROS inhibitors, including TTFA, CCCP, and overexpression of UCP-1 or MnSOD, completely inhibited hyperglycemia-induced PKC activation in the membrane fraction, intracellular AGEs formation, and sorbitol accumulation (59). The same mechanism is also responsible for abnormal activation of the hexosamine pathway in bovine aortic endothelial cells (17). In addition, Brownlee *et al.* (16) showed that hyperglycemia-induced mitochondrial ROS production activates the four major pathways of hyperglycemic damage found in bovine aortic endothelial cells by inhibiting glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity, and this GAPDH inhibition is a consequence of poly(ADP-ribosyl)ation of GAPDH by poly (ADP-ribose) polymerase (PARP), which is activated by DNA strand breaks produced by mitochondrial ROS production. Therefore, a single hyperglycemia-induced process of mitochondrial ROS overproduction may exist as an upstream event of the other pathways (11).

MITOCHONDRIAL ROS PRODUCTION IN TYPE 2 DIABETES PATIENTS

It is still unclear whether mitochondrial ROS production associates with the progression of diabetic complications in type 2 diabetes patients. Therefore, to determine the role of mitochondrial ROS production in type 2 diabetes patients, the relation between 8-hydroxydeoxyguanosine (8-OHdG),

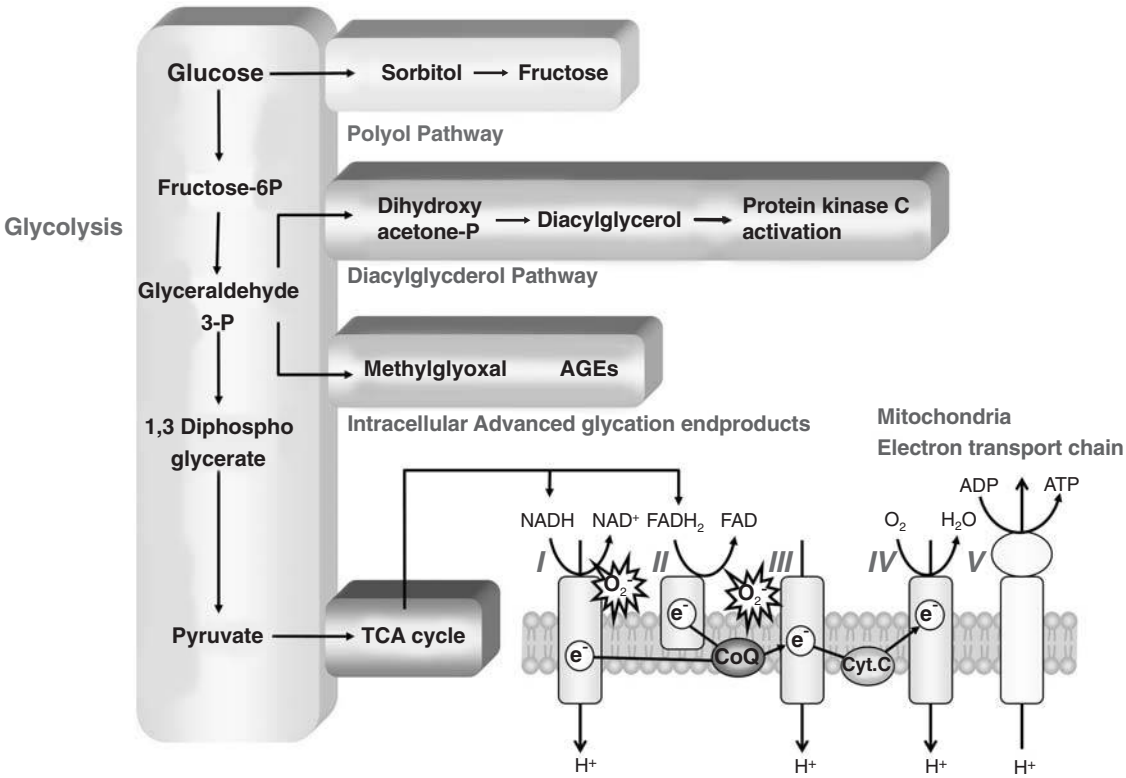

FIG. 2. Glucose metabolism coupled to three major pathways of hyperglycemic damage and mitochondrial reactive oxygen species (ROS) production. The main hypotheses about how hyperglycemia causes diabetic complications are activation of protein kinase C (PKC); increased advanced glycation end products (AGE) formation; activation of polyol pathway; and mitochondrial ROS production. Hyperglycemia-induced mitochondrial ROS overproduction may exist as an upstream event of the other pathways (11).

which is a product of oxidative DNA damage after specific enzymatic cleavage after 8-hydroxylation of the guanine base, and the severity of diabetic complications was examined. As intracellular ROS can cause strand breaks in DNA and base modifications, 8-OHdG is suggested to serve as a sensitive biomarker of intracellular oxidative stress *in vivo* (3, 18, 47, 57). In addition, it has been reported that 8-OHdG was 16-fold higher in mitochondria DNA than in nuclear DNA in rat liver (7). Therefore, 8-OHdG could be a useful biomarker to evaluate ROS production from mitochondria *in vivo*.

8-OHdG in the urine and that of the isolated mononuclear cells from type 2 diabetes patients with either retinopathy or nephropathy were reported to be much higher than those in patients without these complications (82). A similar result was observed in our results of the association between 8-OHdG and either diabetic retinopathy or nephropathy. In addition, the urinary 8-OHdG excretion was elevated in the patients with mean intima–media thickness (IMT) of carotid arteries >1.1 mm, which is considered to be one of the useful markers of atherosclerosis (60). These results suggest that mitochondrial ROS production estimated by the urinary 8-OHdG excretion could associate with both microangiopathy and macroangiopathy in patients with type 2 diabetes.

RELATION BETWEEN GLYCEMIC CONTROL AND MITOCHONDRIAL ROS PRODUCTION

The Kumamoto Study was a randomized clinical trial designed to compare intensive insulin therapy (MIT) using multiple insulin injections with conventional insulin injection therapy (CIT) and to evaluate the effects of glycemic control on the development and progression of microvascular complications in Japanese patients with type 2 diabetes. In the Kumamoto Study, we reported that intensive glycemic control could delay the onset and progression of early stages of diabetic microvascular complications (62, 77, 92). To evaluate the relation between glycemic control and mitochondrial ROS production, urinary 8-OHdG excretion in patients from the Kumamoto Study was measured. 8-OHdG was significantly lower in the MIT group compared with the CIT group, after 10 years of insulin therapy. In addition, after 10-year insulin therapy, the mean IMT was significantly thinner in the MIT group compared with the CIT group (60). Although the content of 8-OHdG and the value of the mean IMT in those patients at commencement of the Kumamoto Study were not measured, these findings suggest that intensive glycemic control could normalize or reduce mitochondrial ROS production, and consequently delay the onset and progression of early stages of diabetic microvascular and macrovascular complications. A recent prospective longitudinal study to assess the progression of nephropathy over 5 years further demonstrated that the urinary 8-OHdG was the strongest predictor of nephropathy among several known risk factors, such as HbA1c, duration of diabetes, and systolic and diastolic blood pressure (33).

POTENTIAL ROLE OF MITOCHONDRIAL ROS PRODUCTION IN DIABETIC NEPHROPATHY

Because it was unclear how mitochondrial ROS production could contribute to the progression of diabetic complications, the impact of mitochondrial ROS on the pathogenesis of diabetic nephropathy was investigated.

Glomerular hyperfiltration is a characteristic finding of early diabetic nephropathy in both human and animal models of diabetes and may play a major role in the pathogenesis (15, 34). The mechanisms mediating an increase in glomerular filtration rate are not well identified, but the vascular reactivity of the renal glomerular efferent arterioles has been shown to be controlled, at least in part, by the release of endogenously synthesized prostaglandins (PGs), allowing autoregulation of glomerular capillary pressure. Because PGs play a role in controlling renal function, it has been proposed that changes in PG production may contribute to the hemodynamic changes observed in diabetes.

Recently, two isoforms of cyclooxygenase (COX), which are the rate-limiting step in biosynthesis of the biologically active and physiologically important PGs, have been identified, COX-1 and COX-2 (78). COX-1 is constitutively expressed in most tissues. In contrast, COX-2 operates as an inducible enzyme with low or undetectable levels in most tissues, and its expression can be markedly increased by a number of inflammatory cytokines, mitogenic factors, and physical stimuli (29, 84). Therefore, we hypothesized that hyperglycemia-induced ROS production through the mitochondrial electron-transport chain could increase the expression of the COX-2 gene and play an important role in diabetes-induced renal hemodynamic alterations.

Incubation with 30 m*M* glucose significantly increased COX-2 mRNA, but not COX-1 mRNA, compared with 5.6 m*M* glucose in cultured human mesangial cells. Similarly, incubation with 30 m*M* glucose significantly increased mitochondrial membrane potential, intracellular ROS production, COX-2 protein expression, and prostaglandin E_2 synthesis, and these events were completely suppressed by TTFA or CCCP, inhibitors of mitochondrial metabolism, or by overexpression of UCP-1 or MnSOD. In addition, hyperglycemia induced activation of the COX-2 gene promoter, which was completely abrogated by mutation of two NF-κB binding sites exist in the COX-2 gene promoter. These results suggest that hyperglycemia increases mitochondrial ROS production, resulting in NF-κB activation, COX-2 gene transcriptional activation, COX-2 mRNA induction, COX-2 protein production, and PGE_2 synthesis (51) (Fig. 3). In several *in vivo* studies, increased immunoreactivity for COX-2 in the renal cortex of streptozotocin-induced diabetic rats (52) and mice (51) was reported. The increased expression of COX-2 protein in renal cortex of diabetic rats was normalized by treatment with insulin (52). In addition, a selective inhibitor of COX-2 significantly decreased the glomerular filtration rate in diabetic rats (52). It should be important to clarify the involvement of mitochondrial ROS production in the pathogenesis of the glomerular hyperfiltration *in vivo*.

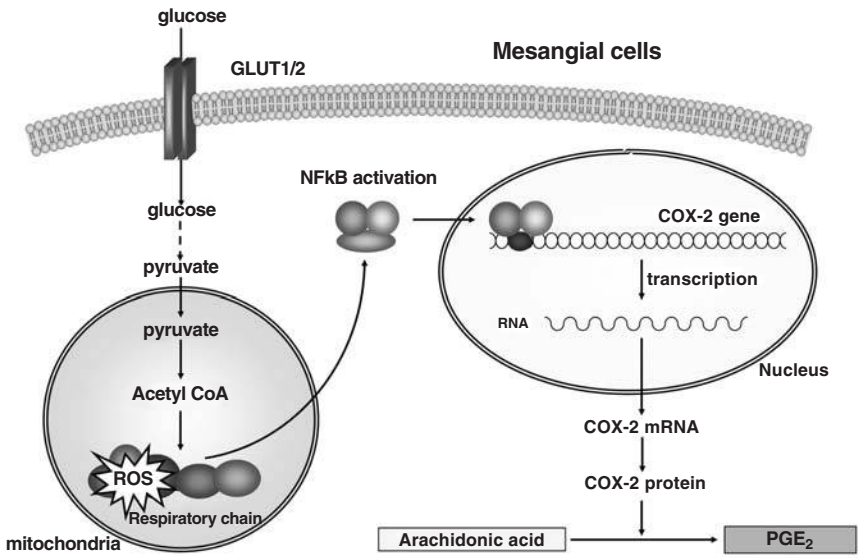

FIG. 3. Potential role of mitochondrial reactive oxygen species (ROS) production in diabetic nephropathy. Hyperglycemia increases mitochondrial ROS production, resulting in NF-κB activation, cyclooxygenase-2 (COX-2) gene transcriptional activation, COX-2 mRNA induction, COX-2 protein production, and PGE_2 synthesis. These chains of events may play a central role in the pathogenesis of the glomerular hyperfiltration observed in early diabetes.

NORMALIZING OF MITOCHONDRIAL ROS PRODUCTION BY ACTIVATION OF AMP-ACTIVATED PROTEIN KINASE (AMPK)

Mitochondrial ROS production therefore seems to be one of the important targets to prevent a progression of diabetic complications. Investigation of molecular factors to reduce mitochondrial ROS production could directly bind to the development of new pharmacologic approaches for prevention of diabetic complications.

In 1998, UKPDS reported intensive glycemic control with metformin, one of the most widely used oral drugs for the treatment of type 2 diabetes, to decrease the risk of diabetes-related end points in overweight patients with type 2 diabetes in comparison to sulfonylurea or insulin therapy (90). Given the equivalent HbA1c levels obtained by every therapy, metformin might have a possible additional benefit in the prevention of diabetic complications independent of its anti-hyperglycemic effect. Conversely, it has been reported that metformin activates AMPK in both hepatocytes and skeletal muscles (103). In addition, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), an AMPK activator, increases the production of peroxisome proliferator activator receptor-γ co-activator-1α (PGC-1α), at least in part, through an AMPK-related mechanism in rat muscle (5, 83). PGC-1α can bind to and coactivate the transcriptional function of nuclear respiratory factors-1 (NRF-1) on the promoter for mitochondrial DNA transcription factor A (mtTFA). mtTFA is a direct regulator of mitochondrial DNA replication/transcription and a stimulus for the regulation of mitochondrial biogenesis and function (96). Therefore, metformin may activate AMPK and induce PGC-1α expression, and these events may affect the hyperglycemia-induced mitochondrial ROS production and mitochondrial biogenesis in endothelial cells.

Treatment with metformin or AICAR inhibited hyperglycemia-induced mitochondrial ROS production, stimulated AMPK activity, and increased the expression of PGC-1α and MnSOD mRNAs in cultured human umbilical vein endothelial cells (HUVECs). The dominant negative form of AMPKα1 (DN-AMPK) diminished the effects of metformin and AICAR on these events, and an overexpression of PGC-1α completely blocked the hyperglycemia-induced mitochondrial ROS production. In addition, metformin and AICAR increased the mRNA expression of NRF-1 and mtTFA and stimulated the mitochondrial proliferation. DN-AMPK also reduced the effects of metformin and AICAR on these observations. These results suggest that metformin normalizes hyperglycemia-induced mitochondrial ROS production by induction of MnSOD through the activation of the AMPK-PGC-1α pathway, and promotes mitochondrial biogenesis through the activation of the same AMPK-PGC-1α pathway (Fig. 4) (54). Thus, AMPK and PGC-1α could be targets for the design of new pharmacologic approaches to prevent diabetic complications.

Recently, mitochondria-targeted therapies using attachment to the lipophilic triphenylphosphonium cation through an alkyl linker was developed (24, 79). These molecules rapidly permeate lipid bilayers and, because of the large mitochondrial membrane potential (negative inside), accumulate several hundredfold inside isolated mitochondria and within mitochondria in cultured cells. Alkyltriphenylphosphonium cations of mitochondria-targeted antioxidants comprising a triphenylphosphonium cation coupled to a coenzyme Q or vitamin E derivative could be fed safely to mice over long periods, coming to steady-state distributions within the heart, brain, liver, and muscle. Therefore, mitochondria-targeted bioactive molecules can be administered orally, leading to their accumulation at potentially therapeutic concentrations in those tissues most affected by mitochondrial dysfunction.

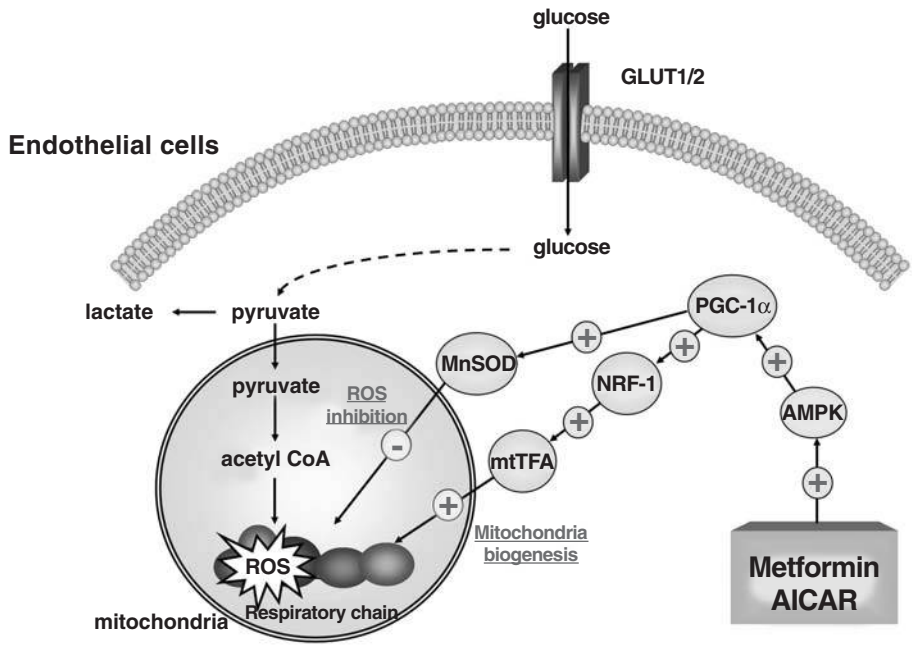

FIG. 4. Inhibitory effect of metformin on mitochondrial reactive oxygen species (ROS) production. Metformin and 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) normalize hyperglycemia-induced mitochondrial ROS production by induction of MnSOD through the activation of the AMP-activated protein kinase (AMPK)-peroxisome proliferator activator receptor-γ coactivator-1α (PGC-1α) pathway. In addition, metformin and AICAR promote mitochondrial biogenesis by induction of nuclear respiratory factors (NRF-1) and mitochondrial DNA transcription factor A (mtTFA) through the activation of the AMPK-PGC-1α pathway.

The mitochondria-specific therapies might be potential therapies for diabetic complications.

IMPACT OF MITOCHONDRIAL ROS PRODUCTION IN GLUCOSE TOXICITY IN PANCREATIC β-CELL

Type 2 diabetes is a polygenic disease aggravated by environmental factors. The development of type 2 diabetes is associated with pancreatic β-cell dysfunction occurring together with insulin resistance. Normal β-cells can compensate for insulin resistance by increasing insulin secretion, but insufficient compensation leads to the onset of glucose intolerance. Once hyperglycemia becomes apparent, β-cell function progressively deteriorates: glucose-induced insulin secretion (GIIS) becomes further impaired, and degranulation of β-cells becomes evident, often accompanied by a decrease in the number of β-cells (14, 48, 69, 101). Although the significance of hyperglycemia as a direct cause of these β-cells dysfunctions, which is called β-cell glucose toxicity, has been demonstrated by various studies *in vivo* (102) and *in vitro* (58, 63, 68, 71, 76), the molecular nature of glucose toxicity in pancreatic β-cell is still unknown.

Conversely, the level of 8-OHdG, a marker for mitochondrial oxidative damage, was reported to be increased in β-cells of diabetic Goto-Kakizaki (GK) rats (43), and antioxidant treatment, *N*-acetyl-L-cysteine (NAC), and vitamins C and E could protect against the onset of diabetes in diabetic *db/db* mice (48). Because antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, are not highly expressed in pancreatic islets compared with other tissues of the body (23, 56, 85), oxidative stress could be involved in glucose toxicity in pancreatic β-cell.

In MIN6 cells, a mouse β-cell line, exposure to high glucose increased intracellular ROS as early as 15 min, and this effect was blunted by TTFA or CCCP. GIIS was also suppressed by H_2O_2, a chemical substitute for ROS. Interestingly, in the perfusion study of the isolated mouse islets, the first phase of GIIS, but not the second phase, was suppressed by 50 μ*M* H_2O_2. Because it was reported that the activities of mitochondrial aconitase, a TCA-cycle enzyme (98), mitochondrial adenine-nucleotide translocase (98), and GAPDH are susceptible to oxidative modification (9, 17), the effects of ROS on GAPDH activity in isolated mouse islets was examined. Because either H_2O_2 or high glucose suppressed the activity of GAPDH, and inhibitors of the mitochondrial function abolished the latter effects, mitochondrial ROS suppressed the first phase of GIIS, at least in part, through the suppression of GAPDH activity. Therefore, mitochondrial overwork, resulting in overproduction of mitochondrial ROS, could be a potential mechanism causing the impaired first phase of GIIS in the early stages of diabetes mellitus (Fig. 5).

IMPACT OF MITOCHONDRIAL ROS PRODUCTION ON INSULIN RESISTANCE

Tyrosine phosphorylation of the insulin-receptor substrates including IRS-1 and IRS-2 through the tyrosine kinase of the insulin receptor is an early key event of the insulin signal transduction (4, 67, 73). Impaired tyrosine phosphorylation of IRS-1 has been reported to involve in various status of insulin resistance *in vivo*.

Insulin resistance is also associated with oxidative stress. Micromolar concentrations of H_2O_2 were reported to inhibit insulin-stimulated tyrosine phosphorylation of IRS-1 (28), and α-lipoic acid, a novel antioxidant, increased insulin-

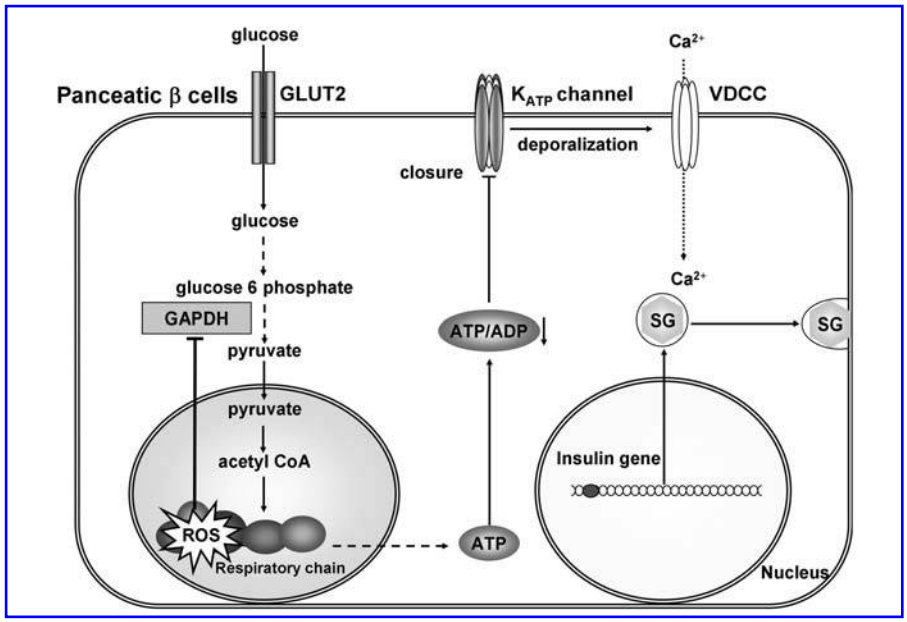

FIG. 5. Potential role of mitochondrial reactive oxygen species (ROS) production in glucose toxicity in pancreatic β-cells. Hyperglycemia-induced mitochondrial ROS production, which suppressed the first phase of glucose-induced insulin secretion through the suppression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity in pancreatic β-cells.

stimulated glucose uptake in muscles (31, 45, 81) and decreased hepatic glucose output (50).

Conversely, it has been suggested that elevation of tumor necrosis factor-alpha (TNF-α) from hypertrophic adipocytes may play a role in causing impaired insulin action (35–37, 39, 49). In addition, similar to hyperglycemia, TNF-α has been reported to increase mitochondrial ROS production in tumor cells (21, 75), hepatocytes (19), and endothelial cells (13). Therefore, roles of mitochondrial ROS in TNF-α–induced impaired insulin signaling in cultured human hepatoma (Huh7) cells were examined.

Using reduced MitoTracker Red probe, TNF-α was confirmed to increase mitochondrial ROS production, and the ROS was suppressed by overexpression of either UCP-1 or MnSOD. TNF-α significantly increased serine phosphorylation of IRS-1, and decreased insulin-stimulated tyrosine phosphorylation of IRS-1, all of which have been considered to be molecular bases for TNF-α–induced insulin resistance (1, 2, 38). All of these observations were inhibited by overexpression of either UCP-1 or MnSOD.

Recently, apoptosis signal-regulating kinase 1 (ASK1) was identified as a mitogen-activated protein kinase kinase kinase (MAPKKK). ASK1 activates the JNK and p38 signaling pathways and is required for TNF-α–induced apoptosis (42). In addition, thioredoxin (Trx), which regulates the cellular reduction/oxidation (redox) status, was reported to bind directly to the N-terminal region of ASK1 (72, 97). Treatment with H_2O_2 induces dissociation of Trx from ASK1, thereby activating ASK1 by inducing oligomerization and the subsequent phosphorylation of a critical threonine residue within the activation loop of ASK1 (22, 72, 86). Therefore, TNF-α may increase mitochondrial ROS production and activate ASK1 in insulin target tissues, and this may cause insulin resistance in diabetes and obesity.

The ASK1 activity in Huh7 cells, measured by an *in vitro* kinase assay with GST-MKK4 as a substrate, was increased as early as 15 min after treatment with TNF-α, and overexpression of either MnSOD or UCP-1 inhibited this effect. Similar to TNF-α, overexpression of wild-type ASK1 increased serine phosphorylation of IRS-1 and decreased insulin-stimulated tyrosine phosphorylation of IRS-1, whereas overexpression of dominant-negative ASK1 ameliorated these TNF-α–induced events. In addition, TNF-α activated c-jun NH_2-terminal kinases (JNK), and this observation was partially inhibited by overexpression of either UCP-1, MnSOD, or dominant-negative ASK1. These results suggest that TNF-α increases mitochondrial ROS production and activates ASK1 in Huh7 cells, and that these TNF-α–induced phenomena contribute, at least in part, to impaired insulin signaling (Fig. 6).

CONCLUSION

Mitochondria are the major source of ROS due to continuously generated superoxide, a byproduct of the electron-transport chain. Here we demonstrated that hyperglycemia-induced mitochondrial ROS production could be a key event in the development of diabetic complications. In addition, we emphasize that mitochondrial ROS production may also be a key factor in the development of type 2 diabetes by induction of impaired insulin secretion and insulin resistance (Fig. 7). The present study provides a conceptual framework for future research and drug discovery, which targets mitochondrial ROS production.

ACKNOWLEDGMENTS

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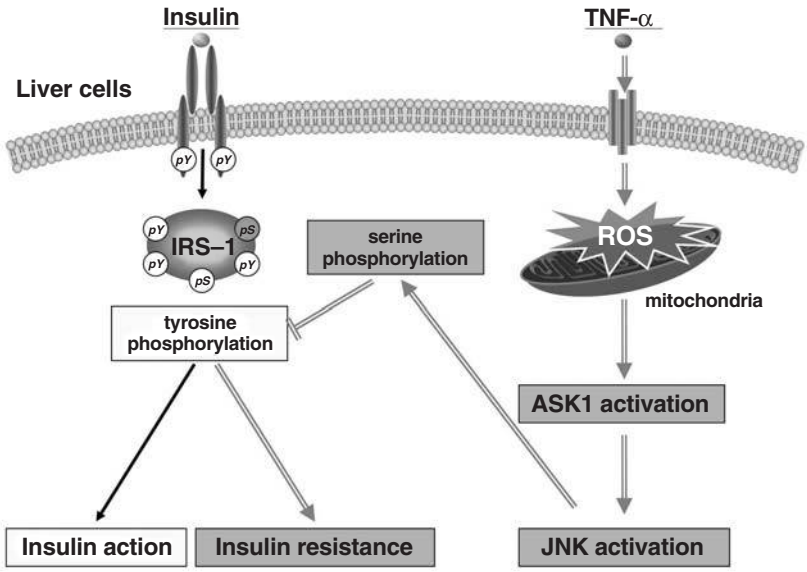

FIG. 6. Potential role of mitochondrial reactive oxygen species (ROS) production in tumor necrosis factor-alpha (TNF-α)-induced insulin resistance. TNF-α increases mitochondrial ROS production, which results in apoptosis signal-regulating kinase 1 (ASK1) activation. In addition, these events activate c-jun NH_2-terminal kinases (JNK), increase serine phosphorylation of insulin receptor substrate-1 (IRS-1), and decrease insulin-stimulated tyrosine phosphorylation of IRS-1, all of which could be involved in the molecular basis of TNF-α–induced insulin resistance.

ABBREVIATIONS

AGEs, advanced glycation end-products; AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside; AMPK, AMP-activated protein kinase**;** ASK1, apoptosis signal-regulating kinase; CCCP, carbonyl cyanide m-chlorophenylhydrazone; CIT, conventional insulin injection therapy; COX, cyclooxygenase; DN-AMPK, dominant negative form of AMPKα1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GIIS, glucose-induced insulin secretion; GK rat, Goto-Kakizaki rat; Huh7 cells, human hepatoma cells; HUVECs, human umbilical vein endothelial cells; IMT, mean intima–media thickness; JNK, c-jun NH_2-terminal kinases; MAPKKK, mitogen-activated protein kinase kinase kinase; MIT, intensive insulin therapy; MnSOD, manganese superoxide dismutase; mtTFA, mitochondrial DNA transcription factor A; NAC. *N*- acetyl-L-cysteine; NRF-1, nuclear respiratory factors-1; 8-OHdG, 8-hydroxydeoxyguanosine; PARP, poly (ADP-ribose) polymerase; PGC-1α, proliferator activator receptor-γ co-activator-1α; PKC, protein kinase C; redox, reduction/oxidation; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha; Trx, thioredoxin; TTFA, thenoyltrifluoroacetone; UCP1, uncoupling protein-1.

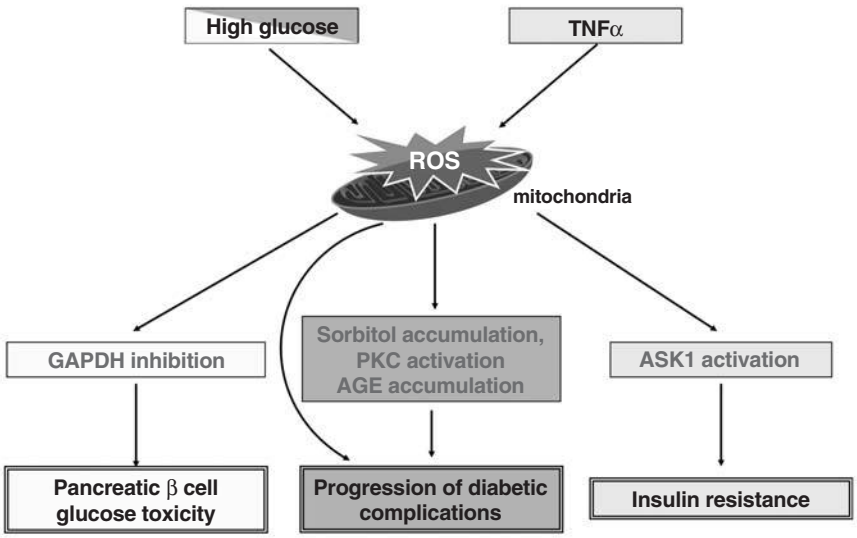

FIG. 7. Impact of mitochondrial reactive oxygen species (ROS) production in diabetes and its complications. Mitochondrial ROS production may be a key factor not only in diabetic vascular complications, but also in the development of pancreatic β-cell glucose toxicity and tumor necrosis factor-alpha (TNF-α)-induced insulin resistance.

REFERENCES

1. Aguirre V, Uchida T, Yenush L, Davis R, and White MF. The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J Biol Chem* 275: 9047–9054, 2000.
2. Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, and White MF. Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. *J Biol Chem* 277: 1531–1537, 2002.
3. Ames BN. Endogenous oxidative DNA damage, aging, and cancer. *Free Radic Res Commun* 7: 121–128, 1989.
4. Araki E, Lipes MA, Patti ME, Bruning JC, Haag B 3rd, Johnson RS, and Kahn CR. Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* 372: 186–190, 1994.
5. Attie AD and Kendziorski CM. PGC-1alpha at the crossroads of type 2 diabetes. *Nat Genet* 34: 244–245, 2003.
6. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 40: 405–412, 1991.
7. Beckman KB and Ames BN. The free radical theory of aging matures. *Physiol Rev* 78: 547–581, 1998.
8. Boveris A and Chance B. The mitochondrial generation of hydrogen peroxide: general properties and effect of hyperbaric oxygen. *Biochem J* 134: 707–716, 1973.
9. Brodie AE and Reed DJ. Reversible oxidation of glyceraldehyde 3-phosphate dehydrogenase thiols in human lung carcinoma cells by hydrogen peroxide. *Biochem Biophys Res Commun* 148: 120–125, 1987.
10. Brownlee M. Advanced protein glycosylation in diabetes and aging. *Annu Rev Med* 46: 223–234, 1995.
11. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 414: 813–820, 2001.
12. Cadenas E, Boveris A, Ragan CI, and Stoppani AO. Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinol-cytochrome c reductase from beef-heart mitochondria. *Arch Biochem Biophys* 180: 248–257, 1977.
13. Corda S, Laplace C, Vicaut E, and Duranteau J. Rapid reactive oxygen species production by mitochondria in endothelial cells exposed to tumor necrosis factor-alpha is mediated by ceramide. *Am J Respir Cell Mol Biol* 24: 762–768, 2001.
14. DeFronzo RA, Bonadonna RC, and Ferrannini E. Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* 15: 318–368, 1992.
15. Ditzel J and Schwartz M. Abnormally increased glomerular filtration rate in short-term insulin-treated diabetic subjects. *Diabetes* 16: 264–267, 1967.
16. Du X, Matsumura T, Edelstein D, Rossetti L, Zsengeller Z, Szabo C, and Brownlee M. Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest* 112: 1049–1057, 2003.
17. Du XL, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, Wu J, and Brownlee M. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc Natl Acad Sci U S A* 97: 12222–12226, 2000.
18. Erhola M, Toyokuni S, Okada K, Tanaka T, Hiai H, Ochi H, Uchida K, Osawa T, Nieminen MM, Alho H, and Kellokumpu-Lehtinen P. Biomarker evidence of DNA oxidation in lung cancer patients: association of urinary 8-hydroxy-2'-deoxyguanosine excretion with radiotherapy, chemotherapy, and response to treatment. *FEBS Lett* 409: 287–291, 1997.
19. Garcia-Ruiz C, Colell A, Mari M, Morales A, and Fernandez-Checa JC. Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species: role of mitochondrial glutathione. *J Biol Chem* 272: 11369–11377, 1997.
20. Giugliano D, Ceriello A, and Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care* 19: 257–267, 1996.
21. Goossens V, Grooten J, De Vos K, and Fiers W. Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. *Proc Natl Acad Sci U S A* 92: 8115–8119, 1995.
22. Gotoh Y and Cooper JA. Reactive oxygen species- and dimerization-induced activation of apoptosis signal-regulating kinase 1 in tumor necrosis factor-alpha signal transduction. *J Biol Chem* 273: 17477–17482, 1998.
23. Grankvist K, Marklund SL, and Taljedal IB. CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse. *Biochem J* 199: 393–398, 1981.
24. Green K, Brand MD, and Murphy MP. Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. *Diabetes* 53(suppl 1): S110–S118, 2004.
25. Ha H, Kim C, Son Y, Chung MH, and Kim KH. DNA damage in the kidneys of diabetic rats exhibiting microalbuminuria. *Free Radic Biol Med* 16: 271–274, 1994.
26. Hall JC. The effect of insulin on intact muscle from normal and alloxan-diabetic rats. *J Biol Chem* 235: 6–8, 1960.
27. Hall JC, Sordahl LA, and Stefko PL. The effect of insulin on oxidative phosphorylation in normal and diabetic mitochondria. *J Biol Chem* 235: 1536–1539, 1960.
28. Hansen LL, Ikeda Y, Olsen GS, Busch AK, and Mosthaf L. Insulin signaling is inhibited by micromolar concentrations of H(2)O(2): evidence for a role of H(2)O(2) in tumor necrosis factor alpha-mediated insulin resistance. *J Biol Chem* 274: 25078–25084, 1999.
29. Harris RC, McKanna JA, Akai Y, Jacobson HR, Dubois RN, and Breyer MD. Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. *J Clin Invest* 94: 2504–2510, 1994.
30. Hayakawa T, Noda M, Yasuda K, Yorifuji H, Taniguchi S, Miwa I, Sakura H, Terauchi Y, Hayashi J, Sharp GW, Kanazawa Y, Akanuma Y, Yazaki Y, and Kadowaki T. Ethidium bromide-induced inhibition of mitochondrial gene transcription suppresses glucose-stimulated insulin release in the mouse pancreatic beta-cell line betaHC9. *J Biol Chem* 273: 20300–20307, 1998.
31. Henriksen EJ, Jacob S, Streeper RS, Fogt DL, Hokama JY, and Tritschler HJ. Stimulation by alpha-lipoic acid of glucose transport activity in skeletal muscle of lean and obese Zucker rats. *Life Sci* 61: 805–812, 1997.
32. Hinokio Y, Suzuki S, Hirai M, Chiba M, Hirai A, and Toyota T. Oxidative DNA damage in diabetes mellitus: its association with diabetic complications. *Diabetologia* 42: 995–998, 1999.
33. Hinokio Y, Suzuki S, Hirai M, Suzuki C, Suzuki M, and Toyota T. Urinary excretion of 8-oxo-7, 8-dihydro-2'-deoxyguanosine as a predictor of the development of diabetic nephropathy. *Diabetologia* 45: 877–882, 2002.
34. Hostetter TH, Troy JL, and Brenner BM. Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int* 19: 410–415, 1981.
35. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, and Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 95: 2409–2415, 1995.
36. Hotamisligil GS, Budavari A, Murray D, and Spiegelman BM. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes: central role of tumor necrosis factor-alpha. *J Clin Invest* 94: 1543–1549, 1994.
37. Hotamisligil GS, Murray DL, Choy LN, and Spiegelman BM. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proc Natl Acad Sci U S A* 91: 4854–4858, 1994.
38. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, and Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 271: 665–668, 1996.
39. Hotamisligil GS, Shargill NS, and Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259: 87–91, 1993.
40. Hunt JV, Bottoms MA, and Mitchinson MJ. Oxidative alterations in the experimental glycation model of diabetes mellitus are due to protein-glucose adduct oxidation: some fundamental differences in proposed mechanisms of glucose oxidation and oxidant production. *Biochem J* 291: 529–535, 1993.

41. Hunt JV, Smith CC, and Wolff SP. Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. *Diabetes* 39: 1420–1424, 1990.
42. Ichijo H, Nishida E, Irie K, ten Dijke P, Saitoh M, Moriguchi T, Takagi M, Matsumoto K, Miyazono K, and Gotoh Y. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 275: 90–94, 1997.
43. Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, Hiai H, Seino Y, and Yamada Y. Hyperglycemia causes oxidative stress in pancreatic beta-cells of GK rats, a model of type 2 diabetes. *Diabetes* 48: 927–932, 1999.
44. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, and Nawata H. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes* 49: 1939–1945, 2000.
45. Jacob S, Streeper RS, Fogt DL, Hokama JY, Tritschler HJ, Dietze GJ, and Henriksen EJ. The antioxidant alpha-lipoic acid enhances insulin-stimulated glucose metabolism in insulin-resistant rat skeletal muscle. *Diabetes* 45: 1024–1029, 1996.
46. Kadowaki T, Kadowaki H, Mori Y, Tobe K, Sakuta R, Suzuki Y, Tanabe Y, Sakura H, Awata T, Goto Y, Hayakawa T, Matsuoka K, Kawamori R, Kamada T, Horai S, Nonaka I, Hagura R, Akanuma Y, and Yazaki Y. A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA. *N Engl J Med* 330: 962–968, 1994.
47. Kaneko T, Tahara S, and Matsuo M. Non-linear accumulation of 8-hydroxy-2'-deoxyguanosine, a marker of oxidized DNA damage, during aging. *Mutat Res* 316: 277–285, 1996.
48. Kaneto H, Kajimoto Y, Miyagawa J, Matsuoka T, Fujitani Y, Umayahara Y, Hanafusa T, Matsuzawa Y, Yamasaki Y, and Hori M. Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. *Diabetes* 48: 2398–2406, 1999.
49. Kanety H, Feinstein R, Papa MZ, Hemi R, and Karasik A. Tumor necrosis factor alpha-induced phosphorylation of insulin receptor substrate-1 (IRS-1): possible mechanism for suppression of insulin-stimulated tyrosine phosphorylation of IRS-1. *J Biol Chem* 270: 23780–23784, 1995.
50. Khamaisi M, Rudich A, Potashnik R, Tritschler HJ, Gutman A, and Bashan N. Lipoic acid acutely induces hypoglycemia in fasting nondiabetic and diabetic rats. *Metabolism* 48: 504–510, 1999.
51. Kiritoshi S, Nishikawa T, Sonoda K, Kukidome D, Senokuchi T, Matsuo T, Matsumura T, Tokunaga H, Brownlee M, and Araki E. Reactive oxygen species from mitochondria induce cyclooxygenase-2 gene expression in human mesangial cells: potential role in diabetic nephropathy. *Diabetes* 52: 2570–2577, 2003.
52. Komers R, Lindsley JN, Oyama TT, Schutzer WE, Reed JF, Mader SL, and Anderson S. Immunohistochemical and functional correlations of renal cyclooxygenase-2 in experimental diabetes. *J Clin Invest* 107: 889–898, 2001.
53. Koya D and King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes* 47: 859–866, 1998.
54. Kukidome D, Nishikawa T, Sonoda K, Imoto K, Fujisawa K, Yano M, Motoshima H, Taguchi T, Matsumura T, and Araki E. Activation of AMP-activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells. *Diabetes* 55: 120–127, 2006.
55. Lee AY, Chung SK, and Chung SS. Demonstration that polyol accumulation is responsible for diabetic cataract by the use of transgenic mice expressing the aldose reductase gene in the lens. *Proc Natl Acad Sci U S A* 92: 2780–2784, 1995.
56. Lenzen S, Drinkgern J, and Tiedge M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free Radic Biol Med* 20: 463–466, 1996.
57. Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K, and Poulsen HE. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis* 13: 2241–2247, 1992.
58. Moran A, Zhang HJ, Olson LK, Harmon JS, Poitout V, and Robertson RP. Differentiation of glucose toxicity from beta cell exhaustion during the evolution of defective insulin gene expression in the pancreatic islet cell line, HIT-T15. *J Clin Invest* 99: 534–539, 1997.
59. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, and Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404: 787–790, 2000.
60. Nishikawa T, Sasahara T, Kiritoshi S, Sonoda K, Senokuchi T, Matsuo T, Kukidome D, Wake N, Matsumura T, Miyamura N, Sakakida M, Kishikawa H, and Araki E. Evaluation of urinary 8-hydroxydeoxy-guanosine as a novel biomarker of macrovascular complications in type 2 diabetes. *Diabetes Care* 26: 1507–1512, 2003.
61. Oberley LW. Free radicals and diabetes. *Free Radic Biol Med* 5: 113–124, 1988.
62. Ohkubo Y, Kishikawa H, Araki E, Miyata T, Isami S, Motoyoshi S, Kojima Y, Furuyoshi N, and Shichiri M. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract* 28: 103–117, 1995.
63. Olson LK, Redmon JB, Towle HC, and Robertson RP. Chronic exposure of HIT cells to high glucose concentrations paradoxically decreases insulin gene transcription and alters binding of insulin gene regulatory protein. *J Clin Invest* 92: 514–519, 1993.
64. Ou P, Nourooz-Zadeh J, Tritschler HJ, and Wolff S. Activation of aldose reductase in rat lens and metal-ion chelation by aldose reductase inhibitors and lipoic acid. *Free Radic Res* 25: 337–346, 1996.
65. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, and Shulman GI. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 300: 1140–1142, 2003.
66. Petersen KF, Dufour S, Befroy D, Garcia R, and Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* 350: 664–671, 2004.
67. Petersen KF and Shulman GI. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *Am J Cardiol* 90: 11G–18G, 2002.
68. Poitout V, Olson LK, and Robertson RP. Chronic exposure of betaTC-6 cells to supraphysiologic concentrations of glucose decreases binding of the RIPE3b1 insulin gene transcription activator. *J Clin Invest* 97: 1041–1046, 1996.
69. Porte D Jr. Banting lecture 1990: Beta-cells in type II diabetes mellitus. *Diabetes* 40: 166–180, 1991.
70. Rinehart RW, Roberson J, and Beattie DS. The effect of diabetes on protein synthesis and the respiratory chain of rat skeletal muscle and kidney mitochondria. *Arch Biochem Biophys* 213: 341–352, 1982.
71. Robertson RP, Zhang HJ, Pyzdrowski KL, and Walseth TF. Preservation of insulin mRNA levels and insulin secretion in HIT cells by avoidance of chronic exposure to high glucose concentrations. *J Clin Invest* 90: 320–325, 1992.
72. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, and Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17: 2596–2606, 1998.
73. Saltiel AR and Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414: 799–806, 2001.
74. Sano T, Umeda F, Hashimoto T, Nawata H, and Utsumi H. Oxidative stress measurement by in vivo electron spin resonance spectroscopy in rats with streptozotocin-induced diabetes. *Diabetologia* 41: 1355–1360, 1998.
75. Schulze-Osthoff K, Bakker AC, Vanhaesebroeck B, Beyaert R, Jacob WA, and Fiers W. Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions: evidence for the involvement of mitochondrial radical generation. *J Biol Chem* 267: 5317–5323, 1992.
76. Sharma A, Olson LK, Robertson RP, and Stein R. The reduction of insulin gene transcription in HIT-T15 beta cells chronically exposed to high glucose concentration is associated with the loss of RIPE3b1 and STF-1 transcription factor expression. *Mol Endocrinol* 9: 1127–1134, 1995.

77. Shichiri M, Kishikawa H, Ohkubo Y, and Wake N. Long-term results of the Kumamoto Study on optimal diabetes control in type 2 diabetic patients. *Diabetes Care* 23(suppl 2): B21–B29, 2000.
78. Sirois J and Richards JS. Purification and characterization of a novel, distinct isoform of prostaglandin endoperoxide synthase induced by human chorionic gonadotropin in granulosa cells of rat preovulatory follicles. *J Biol Chem* 267: 6382–6388, 1992.
79. Smith RA, Porteous CM, Gane AM, and Murphy MP. Delivery of bioactive molecules to mitochondria in vivo. *Proc Natl Acad Sci U S A* 100: 5407–5412, 2003.
80. Sohal RS and Sohal BH. Hydrogen peroxide release by mitochondria increases during aging. *Mech Ageing Dev* 57: 187–202, 1991.
81. Streeper RS, Henriksen EJ, Jacob S, Hokama JY, Fogt DL, and Tritschler HJ. Differential effects of lipoic acid stereoisomers on glucose metabolism in insulin-resistant skeletal muscle. *Am J Physiol* 273: E185–E191, 1997.
82. Suzuki S, Hinokio Y, Komatu K, Ohtomo M, Onoda M, Hirai S, Hirai M, Hirai A, Chiba M, Kasuga S, Akai H, and Toyota T. Oxidative damage to mitochondrial DNA and its relationship to diabetic complications. *Diabetes Res Clin Pract* 45: 161–168, 1999.
83. Terada S, Goto M, Kato M, Kawanaka K, Shimokawa T, and Tabata I. Effects of low-intensity prolonged exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. *Biochem Biophys Res Commun* 296: 350–354, 2002.
84. Tetsuka T, Daphna-Iken D, Miller BW, Guan Z, Baier LD, and Morrison AR. Nitric oxide amplifies interleukin 1-induced cyclooxygenase-2 expression in rat mesangial cells. *J Clin Invest* 97: 2051–2056, 1996.
85. Tiedge M, Lortz S, Drinkgern J, and Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes* 46: 1733–1742, 1997.
86. Tobiume K, Saitoh M, and Ichijo H. Activation of apoptosis signal-regulating kinase 1 by the stress-induced activating phosphorylation of pre-formed oligomer. *J Cell Physiol* 191: 95–104, 2002.
87. Tomita M, Mukae S, Geshi E, Umetsu K, Nakatani M, and Katagiri T. Mitochondrial respiratory impairment in streptozotocin-induced diabetic rat heart. *Jpn Circ J* 60: 673–682, 1996.
88. Tsuruzoe K, Araki E, Furukawa N, Shirotani T, Matsumoto K, Kaneko K, Motoshima H, Yoshizato K, Shirakami A, Kishikawa H, Miyazaki J, and Shichiri M. Creation and characterization of a mitochondrial DNA-depleted pancreatic beta-cell line: impaired insulin secretion induced by glucose, leucine, and sulfonylureas. *Diabetes* 47: 621–631, 1998.
89. Turrens JF and Boveris A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem J* 191: 421–427, 1980.
90. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet* 352: 854–865, 1998.
91. Vester JW and Stadie WC. Studies of oxidative phosphorylation by hepatic mitochondria from the diabetic cat. *J Biol Chem* 227: 669–676, 1957.
92. Wake N, Hisashige A, Katayama T, Kishikawa H, Ohkubo Y, Sakai M, Araki E, and Shichiri M. Cost-effectiveness of intensive insulin therapy for type 2 diabetes: a 10-year follow-up of the Kumamoto study. *Diabetes Res Clin Pract* 48: 201–210, 2000.
93. Williamson JR, Chang K, Frangos M, Hasan KS, Ido Y, Kawamura T, Nyengaard JR, van den Enden M, Kilo C, and Tilton RG. Hyperglycemic pseudohypoxia and diabetic complications. *Diabetes* 42: 801–813, 1993.
94. Wolff SP, Jiang ZY, and Hunt JV. Protein glycation and oxidative stress in diabetes mellitus and ageing. *Free Radic Biol Med* 10: 339–352, 1991.
95. Wollheim CB. Beta-cell mitochondria in the regulation of insulin secretion: a new culprit in type II diabetes. *Diabetologia* 43: 265–277, 2000.
96. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, and Spiegelman BM. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98: 115–124, 1999.
97. Yamawaki H, Haendeler J, and Berk BC. Thioredoxin: a key regulator of cardiovascular homeostasis. *Circ Res* 93: 1029–1033, 2003.
98. Yan LJ, Levine RL, and Sohal RS. Oxidative damage during aging targets mitochondrial aconitase. *Proc Natl Acad Sci U S A* 94: 11168–11172, 1997.
99. Yan LJ, and Sohal RS. Mitochondrial adenine nucleotide translocase is modified oxidatively during aging. *Proc Natl Acad Sci U S A* 95: 12896–12901, 1998.
100. Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, and Stern D. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem* 269: 9889–9897, 1994.
101. Yki-Jarvinen H. Glucose toxicity. *Endocr Rev* 13: 415–431, 1992.
102. Zangen DH, Bonner-Weir S, Lee CH, Latimer JB, Miller CP, Habener JF, and Weir GC. Reduced insulin, GLUT2, and IDX-1 in beta-cells after partial pancreatectomy. *Diabetes* 46: 258–264, 1997.
103. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, and Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 108: 1167–1174, 2001.

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2. Chao Liu, Jiliang Wu, Ming-Hui Zou. 2012. Activation of AMP-activated protein kinase alleviates High-glucose-induced dysfunction of brain microvascular endothelial cell tight-junction dynamics. *Free Radical Biology and Medicine* **53**:6, 1213-1221. [CrossRef]
3. Xiulan Chen, Shasha Wei, Fuquan Yang. 2012. Mitochondria in the pathogenesis of diabetes: a proteomic view. *Protein & Cell* **3**:9, 648-660. [CrossRef]
4. N. Lin, H. Zhang, Q. Su. 2012. Advanced glycation end-products induce injury to pancreatic beta cells through oxidative stress. *Diabetes & Metabolism* **38**:3, 250-257. [CrossRef]
5. Nan Mu, Yuanxi Zhu, Yingmei Wang, Huiying Zhang, Fengxia Xue. 2012. Insulin resistance: A significant risk factor of endometrial cancer. *Gynecologic Oncology* . [CrossRef]
6. Bhaskar Ponugoti, Guangyu Dong, Dana T. Graves. 2012. Role of Forkhead Transcription Factors in Diabetes-Induced Oxidative Stress. *Experimental Diabetes Research* **2012**, 1-7. [CrossRef]
7. Eun Jung Kwak, Young Soon Lee, Eun Mi Choi. 2012. Effect of Magnolol on the Function of Osteoblastic MC3T3-E1 Cells. *Mediators of Inflammation* **2012**, 1-7. [CrossRef]
8. Meenal Pangare, Ayako Makino. 2012. Mitochondrial function in vascular endothelial cell in diabetes. *Journal of Smooth Muscle Research* **48**:1, 1-26. [CrossRef]
9. Luciane B. Ceretta, Gislaine Z. Réus, Helena M. Abelaira, Karine F. Ribeiro, Giovanni Zappellini, Francine F. Felisbino, Amanda V. Steckert, Felipe Dal-Pizzol, João Quevedo. 2012. Increased Oxidative Stress and Imbalance in Antioxidant Enzymes in the Brains of Alloxan-Induced Diabetic Rats. *Experimental Diabetes Research* **2012**, 1-8. [CrossRef]
10. Gordon Fisher, Jessica A. Alvarez, Amy C. Ellis, Wesley M. Granger, Fernando Ovalle, Chiara Dalla Man, Claudio Cobelli, Barbara A. Gower. 2011. Race Differences in the Association of Oxidative Stress With Insulin Sensitivity in African- and European-American Women. *Obesity* . [CrossRef]
11. Dongsheng Cai, Tiewen Liu. 2011. Hypothalamic inflammation: a double-edged sword to nutritional diseases. *Annals of the New York Academy of Sciences* **1243**:1, E1-E39. [CrossRef]
12. Eun Mi Choi. 2011. Liquiritigenin isolated from Glycyrrhiza uralensis stimulates osteoblast function in osteoblastic MC3T3-E1 cells. *International Immunopharmacology* . [CrossRef]
13. Hong Yang, Michael D Nyby, Yan Ao, Ai Chen, David W Adelson, Victoria Smutko, Janake Wijesuriya, Vay Liang W Go, Michael L Tuck. 2011. Role of brainstem thyrotropin-releasing hormone-triggered sympathetic overactivation in cardiovascular mortality in type 2 diabetic Goto–Kakizaki rats. *Hypertension Research* . [CrossRef]
14. Eun Mi Choi. 2011. Deoxyactein stimulates osteoblast function and inhibits bone-resorbing mediators in MC3T3-E1 cells. *Journal of Applied Toxicology* n/a-n/a. [CrossRef]
15. I-Min Liu, Thing-Fong Tzeng, Shorong-Shii Liou, Chia Ju Chang. 2011. Angelica Acutiloba Root Alleviates Advanced Glycation End-Product-Mediated Renal Injury in Streptozotocin-Diabetic Rats. *Journal of Food Science* no-no. [CrossRef]
16. Subhrojit Sen, Shali Chen, Biao Feng, Yuexiu Wu, Edmund Lui, Subrata Chakrabarti. 2011. American ginseng (Panax quinquefolius) prevents glucose-induced oxidative stress and associated endothelial abnormalities. *Phytomedicine* . [CrossRef]
17. Germaine Escames, Luis Carlos López, José Antonio García, Laura García-Corzo, Francisco Ortiz, Darío Acuña-Castroviejo. 2011. Mitochondrial DNA and inflammatory diseases. *Human Genetics* . [CrossRef]
18. Zhong-Wei Zhang, Jian Cheng, Fei Xu, Yang-Er Chen, Jun-Bo Du, Ming Yuan, Feng Zhu, Xiao-Chao Xu, Shu Yuan. 2011. Red blood cell extrudes nucleus and mitochondria against oxidative stress. *IUBMB Life* **63**:7, 560-565. [CrossRef]
19. Sergey Dikalov. 2011. Cross talk between mitochondria and NADPH oxidases. *Free Radical Biology and Medicine* . [CrossRef]
20. I. B. Zavodnik, I. K. Dremza, E. A. Lapshina, V. T. Cheshchevik. 2011. Diabetes mellitus: Metabolic effects and oxidative stress. *Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology* **5**:2, 101-110. [CrossRef]
21. Jingqi Yan, Ziyan Zhang, Honglian Shi. 2011. HIF-1 is involved in high glucose-induced paracellular permeability of brain endothelial cells. *Cellular and Molecular Life Sciences* . [CrossRef]

22. Young Soon Lee, Eun Mi Choi. 2011. Apocynin stimulates osteoblast differentiation and inhibits bone-resorbing mediators in MC3T3-E1 cells. *Cellular Immunology* . [CrossRef]
23. Patrick A. Rowe, Kylie Kavanagh, Li Zhang, H. James Harwood Jr., Janice D. Wagner. 2011. Short-term hyperglycemia increases arterial superoxide production and iron dysregulation in atherosclerotic monkeys. *Metabolism* . [CrossRef]
24. Mário Raimundo, José António Lopes. 2011. Metabolic Syndrome, Chronic Kidney Disease, and Cardiovascular Disease: A Dynamic and Life-Threatening Triad. *Cardiology Research and Practice* **2011**, 1-16. [CrossRef]
25. Kazunori Sango, Hiroko Yanagisawa, Shizuka Takaku, Emiko Kawakami, Kazuhiko Watabe. 2011. Immortalized Adult Rodent Schwann Cells as In Vitro Models to Study Diabetic Neuropathy. *Experimental Diabetes Research* **2011**, 1-9. [CrossRef]
26. Douglas Popp Marin, Anaysa Paola Bolin, Rita de Cássia Santos Macedo, Sandra Coccuzzo Sampaio, Rosemari Otton. 2011. ROS production in neutrophils from alloxan-induced diabetic rats treated in vivo with astaxanthin#. *International Immunopharmacology* **11**:1, 103-109. [CrossRef]
27. Seul Ki Lim, Min Jung Park, Jae Cheong Lim, Jong Chun Kim, Ho Jae Han, Gye-Yeop Kim, Benjamin F. Cravatt, Chang Hoon Woo, Seung Jin Ma, Kyung Cheol Yoon, Soo Hyun Park. 2011. Hyperglycemia induces apoptosis via CB1 activation through the decrease of FAAH 1 in retina pigment epithelial cells. *Journal of Cellular Physiology* n/a-n/a. [CrossRef]
28. Majid Asiabanha, Gholamreza Asadikaram, Amir Rahnema, Mehdi Mahmoodi, Gholamhosein Hasanshahi, Mohammad Hashemi, Mohammad Khaksari. 2011. Chronic Opium Treatment Can Differentially Induce Brain and Liver Cells Apoptosis in Diabetic and Non-diabetic Male and Female Rats. *The Korean Journal of Physiology and Pharmacology* **15**:6, 327. [CrossRef]
29. Sangbin Lim, Md Abdur Rashid, Miran Jang, Yeonghwan Kim, Hyeran Won, Jeonghoon Lee, Jeong-taek Woo, Young Seol Kim, Michael P. Murphy, Liaquat Ali, Joohun Ha, Sung Soo Kim. 2011. Mitochondria-targeted Antioxidants Protect Pancreatic #-cells against Oxidative Stress and Improve Insulin Secretion in Glucotoxicity and Glucolipotoxicity. *Cellular Physiology and Biochemistry* **28**:5, 873-886. [CrossRef]
30. Ki Cheon Kim, Jin Sook Kim, Kyoung Ah Kang, Jong Min Kim, Jin Won Hyun. 2010. Cytoprotective effects of catechin 7-O-#-D glucopyranoside against mitochondrial dysfunction damaged by streptozotocin in RINm5F cells. *Cell Biochemistry and Function* **28**:8, 651-660. [CrossRef]
31. Carlotta Giorgi , Chiara Agnoletto , Claudio Baldini , Angela Bononi , Massimo Bonora , Saverio Marchi , Sonia Missiroli , Simone Patergnani , Federica Poletti , Alessandro Rimessi , Barbara Zavan , Paolo Pinton . 2010. Redox Control of Protein Kinase C: Cell- and Disease-Specific Aspects. *Antioxidants & Redox Signaling* **13**:7, 1051-1085. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
32. Chan Hum Park, Jeong Sook Noh, Noriko Yamabe, Ki Sung Kang, Takashi Tanaka, Takako Yokozawa. 2010. Beneficial effect of 7-O-galloyl-d-sedoheptulose on oxidative stress and hepatic and renal changes in type 2 diabetic db/db mice. *European Journal of Pharmacology* **640**:1-3, 233-242. [CrossRef]
33. Wei Yi, Yang Sun, Xufeng Wei, Chunhu Gu, Xiaochao Dong, Xiaojun Kang, Shuzhong Guo, Kefeng Dou. 2010. Proteomic profiling of human bone marrow mesenchymal stem cells under shear stress. *Molecular and Cellular Biochemistry* **341**:1-2, 9-16. [CrossRef]
34. Wu Li, Yong-Hui Shi, Rui-li Yang, Jue Cui, Ying Xiao, Bin Wang, Guo-Wei Le. 2010. Effect of somatostatin analog on high-fat diet-induced metabolic syndrome: Involvement of reactive oxygen species. *Peptides* **31**:4, 625-629. [CrossRef]
35. K. A. Kang, J. S. Kim, R. Zhang, M. J. Piao, Y. H. Maeng, M. Y. Kang, I. K. Lee, B. J. Kim, J. W. Hyun. 2010. KIOM-4 Protects against Oxidative Stress-induced Mitochondrial Damage in Pancreatic -cells via its Antioxidant Effects. *Evidence-based Complementary and Alternative Medicine* . [CrossRef]
36. K. Schroder, R. Zhou, J. Tschopp. 2010. The NLRP3 Inflammasome: A Sensor for Metabolic Danger?. *Science* **327**:5963, 296-300. [CrossRef]
37. Xiongzhong RUAN, Youfei GUAN. 2009. Metabolic syndrome and chronic kidney disease. *Journal of Diabetes* **1**:4, 236-245. [CrossRef]
38. Ian F. Godsland. 2009. Insulin resistance and hyperinsulinaemia in the development and progression of cancer. *Clinical Science* **118**:5, 315-332. [CrossRef]
39. W Malorni, M G Farrace, P Matarrese, A Tinari, L Ciarlo, P Mousavi-Shafaei, M D'Eletto, G Di Giacomo, G Melino, L Palmieri, C Rodolfo, M Piacentini. 2009. The adenine nucleotide translocator 1 acts as a type 2 transglutaminase substrate: implications for mitochondrial-dependent apoptosis. *Cell Death and Differentiation* **16**:11, 1480-1492. [CrossRef]

40. Lucia Gaddini, Marika Villa, Andrea Matteucci, Cinzia Mallozzi, Tamara C. Petrucci, Anna Maria M. Di Stasi, Lanfranco Leo, Fiorella Malchiodi-Albedi, Flavia Pricci. 2009. Early effects of high glucose in retinal tissue cultures. *Neurobiology of Disease* **35**:2, 278-285. [CrossRef]
41. Fengxia Liang, Shinji Kume, Daisuke Koya. 2009. SIRT1 and insulin resistance. *Nature Reviews Endocrinology* **5**:7, 367-373. [CrossRef]
42. I. R. Sweet, M. Gilbert, E. Maloney, D. M. Hockenbery, M. W. Schwartz, F. Kim. 2009. Endothelial inflammation induced by excess glucose is associated with cytosolic glucose 6-phosphate but not increased mitochondrial respiration. *Diabetologia* **52**:5, 921-931. [CrossRef]
43. Vsevolod A. Tkachuk, Olga S. Plekhanova, Yelena V. Parfyonova. 2009. Regulation of arterial remodeling and angiogenesis by urokinase-type plasminogen activatorThis article is one of a selection of papers from the NATO Advanced Research Workshop on Translational Knowledge for Heart Health (published in part 2 of a 2-part Special Issue). *Canadian Journal of Physiology and Pharmacology* **87**:4, 231-251. [CrossRef]
44. Meriem Mahrouf-Yorgov, Nicolas Marie, Didier Borderie, Raja Djelidi, Dominique Bonnefont-Rousselot, Alain Legrand, Jean-Louis Beaudeux, Jacqueline Peynet. 2009. Metformin suppresses high glucose–induced poly(adenosine diphosphate–ribose) polymerase overactivation in aortic endothelial cells. *Metabolism* **58**:4, 525-533. [CrossRef]
45. Savita Khanna , Han-A Park , Chandan K. Sen , Trimurtulu Golakoti , Krishanu Sengupta , Somepalli Venkateswarlu , Sashwati Roy . 2009. Neuroprotective and Antiinflammatory Properties of a Novel Demethylated Curcuminoid. *Antioxidants & Redox Signaling* **11**:3, 449-468. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
46. V. Di Stefano, C. Cencioni, G. Zaccagnini, A. Magenta, M. C. Capogrossi, F. Martelli. 2009. p66ShcA modulates oxidative stress and survival of endothelial progenitor cells in response to high glucose. *Cardiovascular Research* . [CrossRef]
47. I.-M. Liu, Thing-Fong Tzeng, Shorong-Shii Liou. 2009. Abelmoschus moschatus (Malvaceae), an aromatic plant, suitable for medical or food uses to improve insulin sensitivity. *Phytotherapy Research* n/a-n/a. [CrossRef]
48. Xiao-Yan Zhao, Guo-Fen Qiao, Bao-Xin Li, Li-Min Chai, Zhe Li, Yan-Jie Lu, Bao-Feng Yang. 2009. HYPOGLYCAEMIC AND HYPOLIPIDAEMIC EFFECTS OF EMODIN AND ITS EFFECT ON L-TYPE CALCIUM CHANNELS IN DYSLIPIDAEMIC-DIABETIC RATS. *Clinical and Experimental Pharmacology and Physiology* **36**:1, 29-34. [CrossRef]
49. K TAKI, R SHIMOZONO, H KUSANO, N SUZUKI, K SHINJO, H EDA. 2008. Apoptosis signal-regulating kinase 1 is crucial for oxidative stress-induced but not for osmotic stress-induced hepatocyte cell death. *Life Sciences* **83**:25-26, 859-864. [CrossRef]
50. Liuji Chen, Ren Na, Mingjun Gu, Adam B. Salmon, Yuhong Liu, Hanyu Liang, Wenbo Qi, Holly Van Remmen, Arlan Richardson, Qitao Ran. 2008. Reduction of mitochondrial H 2 O 2 by overexpressing peroxiredoxin 3 improves glucose tolerance in mice. *Aging Cell* **7**:6, 866-878. [CrossRef]
51. C. García-Cáceres, A. Lechuga-Sancho, J. Argente, L. M. Frago, J. A. Chowen. 2008. Death of Hypothalamic Astrocytes in Poorly Controlled Diabetic Rats is Associated with Nuclear Translocation of Apoptosis Inducing Factor. *Journal of Neuroendocrinology* **20**:12, 1348-1360. [CrossRef]
52. T. Jin. 2008. The WNT signalling pathway and diabetes mellitus. *Diabetologia* **51**:10, 1771-1780. [CrossRef]
53. Lisa M Nilsson, Jenny Nilsson-Öhman, Anna V Zetterqvist, Maria F Gomez. 2008. Nuclear factor of activated T-cells transcription factors in the vasculature: the good guys or the bad guys?. *Current Opinion in Lipidology* **19**:5, 483-490. [CrossRef]
54. Savita Khanna, Han-A Park, Chandan K. Sen, Trimurtulu Golakoti, Krishanu Sengupta, Somepalli Venkateswarlu, SASHWATI ROY. 2008. Neuroprotective and anti-inflammatory properties of a novel demethylated curcuminoid. *Antioxidants & Redox Signaling* **0**:ja, 080910041331150. [CrossRef]
55. Hoe Kyu Kim, Yeon Jeong Kim, Jong Tae Kim, Chae Hwa Kwon, Yong Keun Kim, Yong Chan Bae, Dong Heon Kim, Jin Sup Jung. 2008. Alterations in the Proangiogenic Functions of Adipose Tissue–Derived Stromal Cells Isolated from Diabetic Rats. *Stem Cells and Development* **17**:4, 669-680. [Abstract] [Full Text PDF] [Full Text PDF with Links]
56. X Li, J Hu, R Zhang, X Sun, Q Zhang, X Guan, J Chen, Q Zhu, S Li. 2008. Urocortin ameliorates diabetic nephropathy in obese db/db mice. *British Journal of Pharmacology* **154**:5, 1025-1034. [CrossRef]
57. R GARCIAMACEDO, F SANCHEZMUNOZ, J ALMANZAPEREZ, G DURANREYES, F ALARCONAGUILAR, M CRUZ. 2008. Glycine increases mRNA adiponectin and diminishes pro-inflammatory adipokines expression in 3T3-L1 cells. *European Journal of Pharmacology* **587**:1-3, 317-321. [CrossRef]
58. José M. Matés, Juan A. Segura, Francisco J. Alonso, Javier Márquez. 2008. Intracellular redox status and oxidative stress: implications for cell proliferation, apoptosis, and carcinogenesis. *Archives of Toxicology* **82**:5, 273-299. [CrossRef]

59. Yuling Zhao, Naihao Lu, Hailing Li, Yan Zhang, Zhonghong Gao, Yuefa Gong. 2008. High glucose induced human umbilical vein endothelial cell injury: involvement of protein tyrosine nitration. *Molecular and Cellular Biochemistry* **311**:1-2, 19-29. [CrossRef]
60. Eszter M. Horváth, Rita Benk#, Domonkos Ger#, Levente Kiss, Csaba Szabó. 2008. Treatment with insulin inhibits poly(ADP-ribose)polymerase activation in a rat model of endotoxemia. *Life Sciences* **82**:3-4, 205-209. [CrossRef]
61. A. Hagelberg, T. Ivert, S. Efendi#, J. Öhrvik, R. E. Anderson. 2008. Insulin glargine improves glycaemic control after coronary surgery in patients with diabetes or pre-diabetes. *Scandinavian Cardiovascular Journal* **42**:1, 71-76. [CrossRef]
62. David L Duffy. 2007. Genetic determinants of diabetes are similarly associated with other immune-mediated diseases. *Current Opinion in Allergy and Clinical Immunology* **7**:6, 468-474. [CrossRef]
63. Birgit Eichhorn, Dobromir Dobrev. 2007. Vascular large conductance calcium-activated potassium channels: Functional role and therapeutic potential. *Naunyn-Schmiedeberg's Archives of Pharmacology* **376**:3, 145-155. [CrossRef]
64. Dr. Eiichi Araki , Jun-Ichi Miyazaki . 2007. Metabolic Disorders in Diabetes Mellitus: Impact of Mitochondrial Function and Oxidative Stress on Diabetes and Its Complications. *Antioxidants & Redox Signaling* **9**:3, 289-291. [Citation] [Full Text PDF] [Full Text PDF with Links]